UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Heyduk et al.	Art Unit	1634
Serial No:	10/539,107	Examiner	Narayan K. Bhat
Filed:	June 15, 2005	Conf. No.	2295
For:	BIOSENSORS FOR DETECTING MACROMOLECULES AND OTHER ANALYTES		

DECLARATION OF DR. TOMASZ HEYDUK UNDER 37 C.F.R. §1.132

I, Tomasz Heyduk, declare and state as follows:

1. I have over 18 years of experience in the field of chemistry and biochemistry. I am currently employed as a professor for St. Louis University, and have worked at St. Louis University since 1992. My educational background includes a Bachelor of Science degree in the chemical sciences awarded by the University of Wroclaw in the year 1979 and a doctorate degree (i.e., PhD) in Chemistry awarded by the Technical University in the year 1986. I have also published over 65 scientific papers and presented numerous abstracts at internationally attended meetings. Attached to this declaration is a copy of my curricula vitae.

2. I am a co-inventor of U.S. Patent Application Serial No. 10/539,107 ('107 application) entitled "Biosensors for Detecting Macromolecules and Other Analytes." Additionally, I am familiar with the sensor presented in the Baez application (US Patent Application Publication No. 2002/0051986). In light of my first hand knowledge of the '107 application and my knowledge of the state of the art at the time of the filing of the application, I state the following:

    a. It is my considered belief that the combination of non-DNA flexible linkers with signaling oligonucleotides that have a free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol in a biosensor of claim 109 of the '107 application produces the unexpected benefit of significantly increased biosensor sensitivity.

        i. This belief is based, in part, on the data in attached Diagram 2. This data was from an experiment that compared the response of two different biosensors specific for C-peptide. The two separate biosensors were combined with their target, namely C-peptide, and the resulting FRET signal was measured over time.

1. The first biosensor utilized signaling oligonucleotides (R^3 and R^7 in Diagram 1) that have a free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol (ATG AGC).
2. The second biosensor used the overlap sequence described in the Baez et al. patent (CGC CCG A; sequence from oligonucleotide constructs T68 and T66, Table 1 of Baez et al. patent; C and F of Diagram 1), and had a free energy of association higher than 8.0 kcal/mol.
3. The experiment was performed using a mixture of 20 nM and 25 nM fluorescein and Cy5 labeled anti-C-peptide antibodies in 20 mM Tris, 100 mM NaCl, 10 mM EDTA and 0.2 mg/ml BSA.

ii. Robust signal was obtained only with the biosensor that comprised signaling oligonucleotides that have a free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol, as required by claim 109.

iii. Essentially no signal was detected when the Baez et al. sequence was used. This is despite the fact that we attached the Baez sequence to the antibody via a non-DNA linker that we know facilitates FRET signaling. Hence, the major difference between the sensors used in this experiment was the free energy of association, and only the sensor that had signaling oligonucleotides with free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol produced a signal.

b. Additionally, it is my considered belief that the signaling oligonucleotide from the Baez application (CGC CCG A; sequence from oligonucleotide constructs T68 and T66, Table 1 of Baez et al. patent; C and F of Diagram 1) does not have a free energy of association within the limits of claim 109 (e.g. within about 5.5 kcal/mol and about 8.0 kcal/mol).

i. For calculating free energies of hybridization I use the program Hyther from the web site of Dr. John SantaLucia Jr. (http://ozone3.chem.wayne.edu/). This is the same person that authored the paper the Office has cited for calculating free energy values. Importantly, the website allows calculations at various salt concentrations.

1. When I use the Hyther program, the ΔG for the Baez sequence (CGC CCG A) in 0.1 M salt at 20°C is 10.43 kcal/mole (11.92 at 1 M salt). Hence, the Baez application does not teach a complementary nucleic acid sequence with a free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol.

c. It is also my considered belief, that using the SantaLucia website, the signaling oligonucleotide sequence ATG AGC used in the experiment detailed at point 2(a) above does have a free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol.

1. When I use the Hyther program, the ΔG for the sequence used in the experiment above (ATG AGC) at 0.1 M salt and 20°C is 5.76 kcal/mole (7 kcal/mole at 1 M salt), which is within the boundaries of claim 109 (e.g. between about 5.5 kcal/mol and about 8.0 kcal/mol).

3. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

T. Heyduk
Tomasz Heyduk

12-30-08
Date